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NITROGEN CONTENT OF THE HUMUS OF ARID SOILS[1]

By FREDERICK J. ALWAY, *Chief, Division of Soils, Agricultural Experiment Station of the University of Minnesota*, and EARL S. BISHOP, *Industrial Fellow, Mellon Institute*

HISTORICAL REVIEW

One of the most generally recognized characteristics of arid soils (16, p. 163; 15, p. 415; 17, p. 72; 13, p. 147)[2] is the high content of nitrogen contained in their humus, the *matière noire* of Grandeau (6, p. 148).

Attention was first called to this by Hilgard and Jaffa (10), who stated (p. 69):

> It thus appears that on the average the humus of the arid soils contains three times as much nitrogen as that of the humid; that in extreme cases the difference goes as high as 6 to 1.

It is somewhat remarkable that so few other investigators have made any attempt to test this generalization in the case of the soils from the arid portions of either this or any of the other continents.

Fulmer (5) determined the humus nitrogen in 53 soils from Washington, a State with winter rains and summer droughts. In the case of two soils from Skagit County, which has an annual precipitation of about 46 inches, he found 10.46 and 12.04 per cent, respectively, of nitrogen in the humus.

Nabokich (14, p. 339) reports six samples from Bessarabia with from 11.1 to 18.9 per cent of nitrogen in the humus. It seems probable, however, that he has confused the use of the term "humus" as employed on the continent of Europe (organic matter of the soil as determined by combustion with copper oxid) with the sense in which it is generally used in this country. However, he makes a direct comparison of the Danubian soils with those of California, as follows:

> In contrast with the soils of the dry steppes of southern Russia, the humus of the borders of the Danube is quite as rich in nitrogen as that of the soils of the steppes of California and Transcaucasia. The alluviums of the Danube are even richer than those of the Arax.[3]

[1] The work reported in this paper was carried out in 1911 at the Nebraska Agricultural Experiment Station, where the authors were, respectively, Chemist and Assistant in Chemistry.

[2] Reference is made by number to "Literature cited," p. 915-916.

[3] Author's translation (14, p. 339).

Southern Bessarabia has an annual precipitation of about 15 inches, most of it falling during the growing season. Such a climate in this country is commonly referred to as "semiarid."

Loughridge (12, p. 87), in a comprehensive study of the distribution of humus in California soils to a depth of 12 feet, made nearly 1,000 determinations of humus nitrogen, stating that—

> of these there were about 64 where the humus was found to contain more than 10 per cent nitrogen, fourteen of these had from 15 to 20 per cent and but five had more than 20 per cent. . . The general average for all the soils, including the marsh lands, is 5.92 per cent for the first foot, 5.60 per cent for the upper three feet and 5.57 per cent for the entire depth of twelve feet.

Our work was an outgrowth of previous investigations in the same laboratory of the humus of semiarid soils. Samples from the semiarid prairies of Canada had been found by Alway and Trumbull (3) and Alway and Vail (4) to show percentages of nitrogen in the humus similar to those in soils from humid regions. In subsequent, as yet unpublished, studies by Alway and Trumbull and by ourselves many surface soils, representing various soil types and the different degrees of aridity found in Nebraska as well as many samples from the semiarid and desert portions of New Mexico and Arizona, were analyzed without finding even one in which the humus contained as much as 10 per cent of nitrogen. The question then naturally arose as to whether we would meet with similar results if we worked with arid soils from a region of winter rains and summer droughts. Having available a small collection of samples of California soils personally collected in 1909 by one of us in connection with another study, we subjected these to analysis. As our analyses were not fully confirmatory of Hilgard's conclusions, we delayed publication of the results, hoping to be able to continue the work with a more extensive series of samples from California. Since then, Loughridge (12) has reported his findings, with which ours are in general agreement. The question as to the conditions under which a high content of nitrogen in the humus is found in arid soils does not appear as yet at all satisfactorily answered. We present our data in the hope that some one more conveniently located for the collection of the necessary samples will take up the study.

The data upon which Hilgard's conclusions were based are given in the Annual Reports of the Agricultural Experiment Station of the University of California from 1884 to 1902. The method used for the determination of humus nitrogen is described by Hilgard (7, p. 247) and Jaffa (11, p. 35): "Two portions of 5 or 10 grams of air-dried soil (depending on richness in humus)" were placed in prepared filters, washed first with dilute (0.5 to 1.0 per cent) hydrochloric acid, until the filtrate gave no reaction for lime and magnesia, and then with distilled water to neutral reaction. Then the one portion was washed with repeated portions of 6 to 7 per cent ammonia solution until the washings became colorless while the other

was similarly treated with a 4 per cent potassium-hydroxid or a 3 per cent sodium-hydroxid solution. The ammonia solution was used for the determination of the humus, while in the other the humus nitrogen was determined by the Kjeldahl method. On the assumption that the same compounds had been dissolved by the two solvents, the percentage of nitrogen in the humus was calculated.

While Hilgard's conclusions were based upon determinations in which the humus was extracted with an alkaline hydroxid solution, he later suggested as an alternative the use of the ammonia solution (8, p. 22), this being concentrated and then mixed with magnesia and boiled before being subjected to the Kjeldahl determination.

The correctness of the assumption that the ammonia solution dissolves the same compounds or the same proportions of the total nitrogen as the alkaline hydroxids is open to serious question. A mere determination of the nitrogen removed by the two solvents does not suffice to decide the question. The ammonia is likely to combine with some of the dissolved organic matter of the soil, with the result that after the concentration of the extract, preliminary to the Kjeldahl digestion, there may still be present some nitrogen derived from the ammonia in addition to that extracted from the soil. The attempt to eliminate any such combined nitrogen by digestion with magnesia previous to the Kjeldahl determination is unsatisfactory, as the magnesia may decompose some of the nitrogen compounds extracted from the soil with the elimination of ammonia. A determination of the organic carbon in both solvents should be made, and if this is not the same the nitrogen in the alkaline hydroxid solution is not to be regarded as that corresponding to the whole of the organic matter dissolved by the ammonia.

EXPERIMENTAL WORK

We have confirmed Hilgard and Jaffa's (10) observation that after prolonged extraction of a soil with either ammonia or alkaline hydroxid solution the other fails to extract any appreciable amount of black material. Using 10-gm. portions of both a semiarid and a humid soil, we treated with a 4 per cent ammonia solution until the washings became colorless, placed the residues together with 500 c. c. of alkaline hydroxid solution in stoppered bottles, shook these at frequent intervals for eight hours, and then allowed them to stand overnight. In the case of potassium hydroxid, we tried concentrations of 64, 32, 16, 8, 4, 2 per cent and of sodium hydroxid of 36, 18, 9, 4.5, 2.25 per cent. In all cases the amount of coloring matter extracted was so small that the humus could not be satisfactorily determined even by the delicate photometric method (2). Accordingly, it seems safe to assume that the ammonia solution removes the dark coloring matter as completely as the alkaline hydroxids. However, there appears no reason for assuming that a definite relation exists between the quantity of this pigment and the

amount of ammonia-soluble matter in a soil. Comparisons of the color of the ammonia extracts with their content of dissolved matter show that this relation is variable for different depths in the same field and for the same depth in different localities (2, p. 13).

The large number of soils referred to above were analyzed, using the ammonia extract and magnesia, without finding any in which the humus contained as much as 10 per cent of nitrogen. A later critical study of the method showed that the results were not reliable, the amount of humus nitrogen found being affected by the extent to which the solution was concentrated before adding magnesia and also by the time of digestion with the latter. One result of this was that, while parallel determinations gave concordant results, those run one after the other, using the same ammonia solution, gave widely varying results.

The extraction of the humus by the Hilgard-Jaffa method (10) in the case of many soils, especially those of very fine texture, is extremely tedious, being in this respect similar to the Hilgard method for the determination of humus, for which in the case of some soils 10 days or even longer is necessary (7, p. 320). For this reason we sought to devise a more expeditious and convenient method. Using two representative soils, one a silt loam from the Nebraska Experiment Station farm containing 2.41 per cent of humus and 0.245 per cent of total nitrogen, and the other a clay loam from Indian Head, Saskatchewan, Canada, with 1.56 per cent of humus and 0.248 per cent of total nitrogen, we tried shaking 10 gm. of dry soil with 500 c. c. of a 4 per cent potassium-hydroxid solution for periods of 0.5, 1, 2.5, 5, 9, 12, and 24 days. During the working portion of the day the glass-stoppered bottles containing the mixtures were shaken at intervals of about one hour. With both soils the amount of nitrogen dissolved ceased to increase at the end of nine days. Repeated extraction of the same soil with fresh alkali solution, which might have given different results, was not tried.

This method was then compared with that of Hilgard and Jaffa (10), using in the case of five arid soils from California (Table I) both a 4 per cent potassium and a 6 per cent sodium-hydroxid solution.

TABLE I.—*Comparison of methods for the determination of humus nitrogen*

Determination.	Humus nitrogen.			Total nitrogen.
	New method.	Hilgard-Jaffa method.		
		With potassium hydroxid.	With sodium hydroxid.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
A. First	0.162	0.150	0.176	
Second	.159	.152	.180	
Third	.....	.153	.169	
Average	.160	.152	.175	0.260
D. First	.023	.021	.025	
Second	.020	.020	.018	
Third	.....	.020	.023	
Average	.021	.020	.022	.031
F. First	.024	.023	.026	
Second	.025	.021	.026	
Third	.....	.020	.....	
Average	.025	.021	.026	.032
I. First	.058	.045	.037	
Second	.064	.046	.038	
Third	.....	.049	.041	
Average	.061	.047	.039	.104
L. First	.047	.034	.030	
Second	.047	.035	.035	
Third	.....	.038	.035	
Average	.047	.036	.033	.070

The results are only fairly concordant, but the extraction of nitrogen was as complete as by the Hilgard-Jaffa method, and for our study this was the most important consideration.

Using this method, employing a 4 per cent potassium-hydroxid solution and shaking at intervals for 9 days, we determined the humus nitrogen in 16 samples of arid soils from California (Table II). The humus was determined by the Hilgard method (1, p. 319). Duplicate and, in most cases, triplicate determinations were made of both the total nitrogen and the humus nitrogen, and duplicate determinations of the humus.

TABLE II.—*Relation of nitrogen to humus in arid soils from California*

Sample No.	Depth.	Location and description of soil.	Humus.	Humus ash.	Total nitrogen.	Humus nitrogen.	Nitrogen in humus.	
							Found.	Maximum possible.
	Inches.		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
A	0–3	Berkeley. Adobe, virgin.	1.71	0.46	0.260	0.160	9.3	15.2
B	0–3	.....do.....	1.19	.25	.233	.119	10.0	19.6
C	0–6	Waterford. Alluvium, cultivated.	.62	.39	.054	.035	5.6	8.7
D	0–6	Ceres. Loam, cultivated.	.31	.25	.031	.021	6.7	10.0
E	0–3	Fresno. Red hog-wallow land, virgin.	.47	.52	.026	.019	4.1	5.5
F	0–6	Clovis. Red land, cultivated.	.29	.18	.032	.025	8.3	11.0
G	0–6	Fresno. Black adobe, cultivated.	1.39	.92	.144	.087	6.3	10.4
H	0–5	Fresno. Dry bog, virgin.	.75	.45	.078	.030	4.0	10.4
I	0–5	Fresno. Dry bog, cultivated.	.84	.29	.104	.061	7.2	12.4
J	0–5	Clovis. Red land, cultivated.	.38	.16	.060	.032	8.8	15.6
K	0–5	.....do.....	.36	.17	.052	.037	10.4	14.4
L	0–6	Delano. Alluvium, cultivated.	.40	.14	.070	.047	11.8	27.5
M	0–6	Delano. Red land, cultivated.	.50	.32	.061	.036	7.5	12.2
N	0–6	.....do.....	.38	.17	.061	.034	9.0	15.1
O	0–2	Delano. Red land, virgin.	1.00	.34	.159	.115	11.5	15.9
P	0–2	Delano. Alluvium, virgin.	1.17	.20	.187	.140	12.0	16.0
Average			.73	.....	.101	.062	8.3	13.1

All the samples, except the two from Berkeley, Cal., were secured in the San Joaquin Valley in the vicinity of Modesto, Fresno, and Delano, where the normal annual precipitation amounts to 10.9, 9.0, and 6.1 inches, respectively. Samples A and B were both taken from the high hill just east of the buildings on the grounds of the University of California. Sample A is a composite of 20 samples from near the summit, and B of the same number from the lighter colored soil to the west, below the summit. Sample C was from a cultivated field east of Modesto and 4 miles west of Waterford. Sample D was from a fallowed field 3 miles south of Hickman and 12 miles east of Ceres, E from the virgin red hog-wallow land 7 miles north of Fresno, and F from the red lands 10 miles east of Clovis. The last-named had been under cultivation from 15 to 20 years. Sample G is a black adobe from the same farm as sample F, and the field had been under crop for about 7 years. Sam-

which F and G were secured. H was from virgin soil, while I was from land which had been formerly cultivated, but allowed to revert to grass about 10 years before. Samples J and K were taken from two fallowed fields of red land about 2 miles east of Clovis. The remaining samples were from near Delanò—L from a field under cultivation for 15 years and M and N from fallows on red land north of the White River.

Of the 16 samples only 5 show as high as 10 per cent of nitrogen in the humus. For the 6 samples of virgin soil the average is 8.5 per cent, with a maximum of 12.0 and a minimum of 4.0 per cent. For the 10 of cultivated soils the corresponding data are 8.1, 11.8, and 5.6 per cent, respectively. The maximum possible percentages of nitrogen in the humus—the relation of the total nitrogen to the humus—ranged from 5.5 to 19.6 per cent, with an average of 13.1. Hilgard (9, p. 424), in a comparison of the average composition of 313 arid and 466 humid soils, reports the former to show 0.75 per cent humus and 15.87 per cent of nitrogen and the latter 2.70 per cent of humus, with only 5.45 per cent of nitrogen.

There is no reason to doubt the reliability of the humus determinations upon which Hilgard's generalizations are based. A careful study (1) has shown that his method, as carried out by himself, gives results strictly comparable with those of the Moores-Hampton method. We have examined the original data on the humus determinations by Hilgard and his assistants and in only a very few cases do we find a humus-ash content sufficiently high to make the determination appear inaccurate. These percentages of humus ash, while not reported in the tables in Hilgard's articles discussing the relation of the nitrogen content of humus to climate, may be found in the original reports referred to above.

In that we found 5 out of 16 arid soils to have over 10 per cent of nitrogen in the humus after having failed to find any humid or semiarid soil with such a high percentage, our study tends to confirm the work of Hilgard that high percentages are to be found in the arid but not in the humid soils. This high nitrogen content of the humus, however, does not appear so general in the arid soils as to serve as an at all reliable means of identification.

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LIFE-HISTORY STUDIES OF THE COLORADO POTATO BEETLE

By PAULINE M. JOHNSON, *Scientific Assistant*, and ANITA M. BALLINGER, *formerly Preparator, Truck-Crop and Stored-Product Insect Investigations, Bureau of Entomology.*

INTRODUCTION

The experiments on the life history of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), the details of which follow, were suggested by Dr. F. H. Chittenden, in charge of Truck-Crop and Stored-Product Insect Investigations of the Bureau of Entomology, and were conducted under his direction. These studies were necessarily carried on indoors for the most part and under somewhat unnatural conditions. Had they been conducted out of doors, the probabilities are that in any well-kept field of potatoes (*Solanum tuberosum*) the beetles would have passed through a period of estivation; and if the potatoes had been grown under weedy conditions, where the beetles had access to wild solanaceous plants, the third generation would have been produced. All experiments were performed in the District of Columbia during the season of 1914. The temperature during the period of the work was exceedingly high, with more than the normal rate of humidity.

GENERATION EXPERIMENTS

The overwintered beetles of this species made their first appearance after hibernation on April 29 on *Solanum jasminoides*, an ornamental plant growing in the insectary garden. Beetles were collected and pairs isolated in jars for experimental purposes. After feeding for a few days the females began depositing their characteristic orange-colored eggs (Pl. LXIII, fig. 1) in masses on the underside of the leaves near the tips. The egg masses averaged from 35 to 45 eggs each, except in two cases observed, in which as many as 70 and 72 eggs, respectively, were counted. When the potato plants first emerged from the ground, the beetles showed a decided preference for them, deserting the foliage of *S. jasminoides* for the more tender leaves of the potato.

The fecundity of single females, under the conditions described, is shown in Tables I to VIII.

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FIRST GENERATION

TABLE I.—*Eggs produced by a single overwintered female of the Colorado potato beetle; male and female taken in copula on April 30, 1914, and placed in rearing jar with growing potato plant*[1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 4....	17	17	May 15...	24	24	May 25...	4	4
5....	0	0	16...	0	0	26...	0	0
6....	0	0	17...	0	0	27...	0	0
7....	0	0	18...	16	16	28...	0	0
8....	43	43	19...	0	0	29...	14	14
9....	0	0	20...	0	0	30...	16	16
10....	67	67	21...	10	10	31...	24	1,5,10,8
11....	31	31	22...	9	9	June 1...	3	3
12....	45	45	23...	0	0	Total..	379	.....
13....	28	28	24...	10	10			
14....	18	18						

[1] The male in this experiment died on June 10, the female on June 14.

TABLE II.—*Eggs produced by a single overwintered female of the Colorado potato beetle; pair collected at College Park, Md., and placed in rearing jar on May 11, 1914, with growing potato plant*[1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11..	78	32, 46	May 31...	70	21, 18, 31	June 19...	0	0
12..	51	31, 20	June 1...	25	25	20...	5	5
13..	31	31	2...	21	21	21...	0	0
14..	0	0	3...	0	0	22...	0	0
15..	0	0	4...	2	2	23...	9	9
16..	0	0	5...	0	0	24...	40	40
17..	90	30, 54	6...	0	0	25...	14	14
18..	0	0	7...	0	0	26...	0	0
19..	36	36	8...	0	0	27...	0	0
20..	32	32	9...	0	0	28...	0	0
21..	34	9, 11, 17	10...	6	0	29...	0	0
22..	0	0	11...	0	0	30...	0	0
23..	0	0	12...	0	0	July 1...	0	0
24..	34	34	13...	0	0	2...	0	0
25..	72	24, 48	14...	0	0	3...	0	0
26..	29	20, 9	15...	34	34	4...	0	0
27..	25	25	16...	0	0	5...	24	24
28..	47	47	17...	71	52, 19	Total..	994	.....
29..	33	33	18...	50	50			
30..	28	28						

[1] The male died on June 7, the female on July 7.

TABLE III.—*Eggs produced by a single overwintered female of the Colorado potato beetle; male and female collected at College Park, Md., and placed in confinement on May 10, 1914, with growing potato plant* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	44	44	May 26...	0	0	June 9...	0	0
12...	16	16	27...	0	0	10...	0	0
13...	0	0	28...	0	0	11...	0	0
14...	0	0	29...	25	25	12...	0	0
15...	46	46	30...	0	0	13...	0	0
16...	0	0	31...	0	0	14...	23	23
17...	39	39	June 1...	23	23	15...	42	42
18...	0	0	2...	16	16	16...	33	33
19...	36	19, 17	3...	0	0	17...	0	0
20...	11	11	4...	0	0	18...	0	0
21...	0	0	5...	0	0	19...	0	0
22...	0	0	6...	0	0	20...	16	16
23...	0	0	7...	10	10			
24...	0	0	8...	0	0	Total.	389	.....
25...	0	0						

[1] The male died on June 23, the female on September 2.

TABLE IV.—*Eggs produced by a single overwintered female of the Colorado potato beetle; pair of adults taken in copulation and isolated in a rearing jar on May 11, 1914, with a growing potato plant* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	57	57	June 4...	56	20, 36	June 28...	0	0
12...	15	15	5...	41	41	29...	0	0
13...	15	15	6...	41	41	30...	33	33
14...	58	58	7...	45	45	July 1...	42	42
15...	0	0	8...	35	35	2...	0	0
16...	54	54	9...	60	60	3...	43	43
17...	57	57	10...	43	43	5...	35	35
18...	0	0	11...	57	57	6...	0	0
19...	33	33	12...	84	19, 65	7...	47	47
20...	25	25	13...	47	47	8...	61	22, 39
21...	21	21	14...	54	54	9...	13	13
22...	31	31	15...	55	55	10...	30	30
23...	34	34	16...	51	51	11...	0	0
24...	0	0	17...	39	39	12...	20	20
25...	56	56	18...	46	34, 12	13...	0	0
26...	26	26	19...	31	31	14...	0	0
27...	27	27	20...	0	0	15...	13	13
28...	0	0	21...	7	7	16...	8	8
29...	37	37	22...	1	1	17...	16	16
30...	42	42	23...	0	0	18...	0	0
31...	30	30	24...	0	0	19...	8	8
June 1...	25	25	25...	0	0	20...	14	14
2...	36	36	26...	0	0			
3...	0	0	27...	0	0	Total.	1,879	.....

[1] The male died on August 1, the female on August 20. In this experiment the duration of egg-laying extended over a period of 70 days, or 10 weeks.

TABLE V.—*Eggs produced by a single overwintered female of the Colorado potato beetle; male and female in copula isolated on May 11, 1914, with growing potato in rearing jar* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 14..	36	36	June 3..	0	0	June 23..	6	6
15..	0	0	4..	39	39	24..	0	0
16..	43	43	5..	45	14, 31	25..	16	16
17..	0	0	6..	43	43	26..	0	0
18..	20	20	7..	35	35	27..	11	11
19..	0	0	8..	46	33, 13	28..	0	0
20..	0	0	9..	54	54	29..	38	38
21..	54	27, 27	10..	63	34, 11, 18	30..	0	0
22..	0	0	11..	62	30, 32	July 1..	0	0
23..	32	32	12..	24	24	2..	20	20
24..	33	33	13..	57	57	3..	8	8
25..	19	19	14..	34	34	4..	0	0
26..	25	25	15..	33	33	5..	0	0
27..	67	33, 34	16..	31	31	6..	0	0
28..	0	0	17..	34	34	7..	0	0
29..	40	40	18..	25	25	8..	4	4
30..	42	42	19..	0	0			
31..	48	12, 12, 24	20..	8	8	Total..	1,301	.....
June 1..	32	32	21..	12	12			
2..	43	43	22..	19	19			

[1] The male died on June 25, the female on July 27. Temperatures: Maximum, 98° F.; minimum, 43° average, 72°.

Eggs which were deposited on May 4 hatched on May 12, and the larvæ (Pl. LXIII, fig. 2) fed ravenously until May 28, when they entered the ground to a depth of about 3 inches and transformed to pupæ on May 30. Adults emerged on June 9.

Eggs which were deposited on May 7 hatched on May 16. The larvæ became full grown, pupated on May 31, and entered the soil, from which the adults issued on June 10.

SECOND GENERATION

After the adults of the first generation had issued from the ground, three pairs were isolated while in copulation and placed in jars with potato leaves as food on June 17, 18, and 19, respectively.

TABLE VI.—*Record of egg deposition of first-generation female of pair 1 of the Colorado potato beetle, confined in rearing jar on June 17, 1914, and fed upon potato foliage* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
June 22..	22	22	July 5...	11	11	July 17...	47	47
23..	45	45	6...	0	0	18...	44	44
24..	11	11	7...	19	19	19...	45	45
25..	23	23	8...	24	24	20...	0	0
26..	0	0	9...	0	0	21...	0	0
27..	0	0	10...	0	0	22...	37	37
28..	44	44	11...	0	0	23...	0	0
29..	2	2	12...	0	0	24...	0	0
30..	15	15	13...	22	22	25...	0	0
July 1..	47	47	14...	0	0	26...	17	17
2..	27	27	15...	0	0	27...	11	11
3..	0	0	16...	0	0	Total..	513	.....
4..	0	0						

[1] The male in this experiment died on June 20, the female on August 4.

The male and female of pair 2, having been confined to the rearing jar on June 18, 1914, fed for a few days upon the potato foliage, after which they entered the ground for hibernation, the female depositing no eggs.

TABLE VII.—*Record of egg deposition of first-generation female of pair 3 of the Colorado potato beetle, confined in rearing jar on June 19, 1914, and fed upon potato foliage* [1]

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
July 1...	4	4	July 10...	0	0	July 18...	38	38
2...	0	0	11...	0	0	19...	18	18
3...	32	32	12...	0	0	20...	38	38
4...	30	30	13...	0	0	21...	0	0
5...	38	38	14...	0	0	22...	15	15
6...	48	48	15...	0	0	23...	65	65
7...	46	46	16...	0	0	Total..	502	.....
8...	33	33	17...	59	22,37			
9...	38	38						

[1] The male of this pair went into hibernation on July 20, the female on July 27. Temperatures: Maximum, 102° F.; minimum, 58°; average, 72°.

A mass of eggs which was deposited on June 30 by the female of pair 1 hatched on July 7. The larvæ became full-grown on July 23, pupated on July 25, and emerged as adults on July 31. Another mass of eggs laid on July 10 by the same female hatched on July 16, the larvæ pupating on August 5 and issuing as adults on August 11.

THIRD GENERATION

When the adults of the second generation had emerged, pairs were isolated as in previous experiments.

TABLE VIII.—*Record of egg deposition of second-generation female of a pair of the Colorado potato beetle, confined in rearing jar and fed upon potato foliage*[1]

Date.	Number of eggs laid.	Number of eggs to a mass.
1914.		
August 20	19	19
21	48	48
22	0	0
23	45	45
Total	112	

[1] Temperatures: Maximum, 96° F.; minimum, 46°; average, 70°.

In the rearing experiments with the third generation the females of the second generation did not all oviposit. Four pairs began hibernation after feeding for several days. One mass of eggs deposited on August 4 hatched on August 9, the larvæ pupating on August 23 and the adults emerging on August 31. Another egg mass, which was deposited on August 21, hatched on August 26, and the larvæ, becoming full-grown on September 14, entered the ground for pupation, the adults emerging on September 23.

All of the beetles of this third generation were very active and fed voraciously on the foliage of the potato up to September 15.

LENGTH OF STAGES

Table IX shows the maximum and minimum number of days covered by each of the immature stages in each of the three generations, as obtained from the foregoing rearing experiments.

TABLE IX.—*Maximum and minimum length (in days) of immature stages of the Colorado potato beetle in each of the three generations*

Generation.	Egg stage.		Larval stage.		Pupal stage.		Total developmental period.	
	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.
First	7	9	15	18	10	10	30	37
Second	6	7	16	18	6	8	32	41
Third	5	5	14	19	8	9	27	35

NUMBER OF MOLTS AND DURATION OF INSTARS

Eggs of the Colorado potato beetle were segregated and watched carefully to determine the number of molts of the larvæ and the time spent in each instar. It was found that every larva has three molts, with an average of about three days for each instar. Tables X and XI show the dates and number of days required for the molts.

TABLE X.—*Number of molts and dates of molting of Colorado potato-beetle larvæ in 1914*

Experiment No.	Egg hatched.	First molt.	Second molt.	Third molt.
1	July 26	July 29	Aug. 2	Aug. 5
2	...do....	...do....	Aug. 1	Aug. 3
3	July 30	Aug. 2	Aug. 4	Aug. 8
4	...do....	...do....	...do....	Do.
5	...do....	...do....	...do....	Aug. 6
6	...do....	...do....	...do....	Aug. 8
7	Aug. 7	Aug. 9	Aug. 15	Aug. 19
8	...do....	Aug. 10	...do....	Do.

TABLE XI.—*Duration (in days) of instars of Colorado potato-beetle larvæ*

Experiment No.	First instar.	Second instar.	Third instar.
1	3	4	3
2	3	3	2
3	3	2	4
4	3	2	4
5	3	2	2
6	3	2	4
7	2	6	4
8	3	5	4
Maximum duration			2
Minimum duration			6

FALL MATING FOR SPRING EGG LAYING

The fact that the Colorado potato beetle may be observed mating in September in the latitude of the District of Columbia has probably given rise to the opinion that a third generation might be produced elsewhere—e.g., in Minnesota. This last generation, whether second or third, has been proved in one instance to be fertilized in the fall, the females on issuing being capable of depositing eggs in the spring without a second copulation. This was found to be the case with the generation which held over from 1914 and was observed in the spring of 1915, for a female came to the surface on March 8 and, without mating, deposited eggs on March 11 and 12, which hatched on March 20 and 21. These larvæ fed until March 30 and 31, when they pupated, the adults emerging on April 19,

1915. This was an indoor experiment, and the beetles had been kept in a warm room during this entire period. In the field the first adults were observed in the insectary garden May 4, 1915. It was quite cold during that period compared with the earlier season of 1914.

SUMMARY AND CONCLUSIONS

In the authors' experiments in 1914 in the District of Columbia eggs of the Colorado potato beetle were laid almost immediately after the first overwintering beetles were collected in copulation in the spring. These overwintering beetles fed continuously until September 7, when the last one died. The adults of the first generation upon emergence fed for a short time; some of them went into hibernation, but most of them laid eggs for a second generation. Likewise, some adults of the second generation hibernated, while others laid eggs from which adults of the third generation developed. Dr. Chittenden has stated [1] that in the course of his investigations he was not able to get the beetle to breed more than twice in a season without a period of estivation; but from the few eggs that were laid in the second generation the authors were able to rear the species through three generations without a resting period.

In 1908 Popenoe [2] made experiments with this insect in tidewater Virginia, and reared it through three generations, but all the beetles of the third generation died. In this experiment the heat was still greater than in Washington in 1914, and the insects were not isolated in large numbers and were not well fed, which accounts for the dying of the third generation.

The entire developmental period from egg to adult was passed, as previously stated by Dr. Chittenden, in approximately four weeks.

Particular attention is called to the fact that the female, far from laying the small number of eggs attributed to this species, is capable of laying, in one case under actual observation, 1,879, while a second female deposited 1,301 eggs. The former record exceeds any hitherto published, so far as known. It should be stated, however, that during 1913 Mr. W. O. Ellis,[3] of the Iowa Agricultural Experiment Station, obtained from a single female of the species a total of 1,686 eggs, and that Messrs. Girault and Zetek [4] took 1,578 eggs from a single beetle.

From the experiments reported herein it is evident that there are three completed generations of the Colorado potato beetle in the District

[1] Chittenden, F. H. The Colorado potato beetle (Leptinotarsa decemlineata Say). U. S. Dept. Agr. Bur. Ent. Circ. 87, p. 8–9. 1907.

[2] Popenoe, C. H. The Colorado potato beetle in Virginia in 1908. U. S. Dept. Agr. Bur. Ent. Bul. 80, pt. 1, 8 p., 2 pl. 1909.

[3] Ellis, W. O. Leptinotarsa decemlineata Say. *In* Jour. Econ. Ent., v. 8, no. 6, p. 520–521. 1915.

[4] Girault, A. A., and Zetek, James. Further biological notes on the Colorado potato beetle, Leptinotarsa 10-lineata (Say), including observations on the number of generations and length of the period of oviposition. I'. Illinois. *In* Ann. Ent. Soc. Amer., v. 4, no. 1, p. 74. 1911.

of Columbia and localities having the same mean temperatures, part of the adults of the first and second generations hibernating, while the remainder lay eggs from which the second and third generations develop. Furthermore, the possibility of a partial fourth generation is suggested by the fact that the beetles of the third generation were active and feeding voraciously during September, 1914. This insect is to be found in all stages during the summer months, and there is much overlapping of generations.

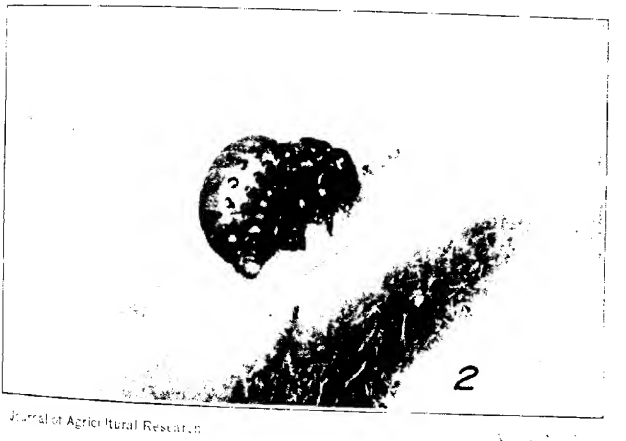
22533°—16——2

PLATE LXIII

Colorado potato beetle (*Leptinotarsa decemlineata*):

Fig. 1.—Egg mass, highly magnified. Original.
Fig. 2.—Young larva, highly magnified. Original.


1


2

like *Streptococcus mesenterioides* the naked modification—i. e., the form developed on a medium containing no sugar and having no capsule—succumbs more quickly as a result of desiccation than does the encapsulated form. *S. mesenterioides* (13, p. 244) has been found to resist desiccation for a much longer period if developed on a saccharin medium than on one which contains no sugar. Revis (20) shows that two types of colon organisms which developed a mucilaginous type of growth were the ones which survived longest in soil. In another article (21) he suggests that the slime formed by organisms of the colon type may add to the water-absorbing and water-retaining capacity of the soil, and may therefore promote the longevity of that organism. Löhnis (15) says that not only the spores but also the bacteria with slimy walls endure the effects of desiccation very well. Lafar (13) emphasizes the importance of making a distinction between organisms like *S. mesenterioides*, which surrounds itself with a gelatinous envelope, and organisms which carry on a slimy fermentation—i. e., conversion of sugar outside the cell into mucinous matter—without themselves being inclosed in capsules. Jensen (11, p. 323) uses the terms capsule formation and slimy fermentation interchangeably and regards the process as protecting the organism against desiccation.

Buchanan (2, p. 378) offers a very comprehensive review of the literature on the nature and morphological origin of bacterial slimes. Some describe gum formation as the result of a true fermentation of carbohydrates by bacteria, calling it an extracellular synthesis, others calling it a true synthetic process, but not necessarily due to an extracellular ferment. Most of the bacterial gums reported in the literature are described as carbohydrates of the formula $(C_6H_{10}O_5)_n$. Bacterial slimes classed as dextrans are described by Bräutigam, Kramer, Ritsert, Scheibler, and many others (2). Lipman, Greig-Smith, Maassen, and Laxa (2) found levulan to be the specific gum of several slime-forming bacteria. Schmidt-Mühlheim, Hueppe, Emmerling, Greig-Smith, Laurent, Ward, and Seiler (2) describe bacterial gums having the characteristics of galactans. A few nitrogenous bacterial gums are mentioned, but they appear to be less common than those of a carbohydrate nature. The protective action of these gums has been ascribed to their water-retaining capacity.

Exclusive of organisms with such special protective structures as spores or capsules, it appears to be true that certain species are more resistant than others. Neisser (4) found that the organisms of typhoid fever and diphtheria were the most resistant; cholera, influenza, bubonic plague, and gonococci the least; and the pus-forming cocci, meningococcus, and tubercle bacillus of intermediate resistance. Briscoe (1) credits the tubercle bacillus with a greater resistance than most non-spore-bearing organisms. This power of resistance is no doubt due in part to the waxy or fatty substance found largely in the outer layer of the tubercle bacillus.

Ficker (8) states that the temperature at which the organisms are cultivated and their ability to resist drying at different temperatures stand in a certain relation. Drying at a higher temperature does not always produce a more rapid effect and the drying at a lower temperature a more gradual effect. He concluded that cultivation at a temperature below the optimum produces an individual with the greatest resistance to desiccation. His results (7) with the drying of cholera vibrio cultures of different ages indicate that cultures 1 or 2 days old endure desiccation better than older cultures, but of these two the 48-hour culture is less sensitive to drying at 37° C. than is the 24-hour culture. The results of Kitasato and Berckholtz, quoted in the same article, show about the same resistance in cultures from 1 to 5 days old. Cultures older than these showed a marked decrease in resistance, due not only to the fact that there were fewer living organisms present in the same mass of an old culture, but these surviving organisms possessed in themselves less vitality than did the vibrios from younger cultures. Ficker (7) also demonstrated in the case of the cholera vibrio that a virulent strain was more resistant than an avirulent strain. Ficker's experiments (8) showed that transfers of old cholera vibrios from the surface of agar to distilled water resulted in a disturbance of the turgor of the cell which was so injurious as to make its death, when desiccated, occur much sooner than was the case when they were suspended in physiological salt solution and dried. With young cultures the reverse was true. Suspension in tap water or distilled water appeared to have the same effect, but desiccation after suspension in physiological salt solution was quickly injurious. He explains this on the basis that since the drying process resulted in an increase of concentration of the salt solution, the cell was subjected to both plasmolysis and desiccation. The explanation is not complete, however, for a broth of the same salt content as the physiological salt solution was favorable to both young and old cultures. He found (8) the cholera vibrio to retain its vitality longer when dried from a suspension in milk or broth than in distilled water, tap water, physiological salt solution, serum, or saliva. Ficker (8) also showed that a greater longevity resulted after drying on cover-glass films when the organisms were first cultivated on a solid medium and then suspended in fresh broth or milk, than when they were grown in those liquids and then dried on cover-glass films prepared directly from the medium in which they developed.

Peiser (17) showed that the thermal death point of lactic-acid bacteria when determined in milk is higher than when determined in bouillon. Numerous examples are cited of the long preservation of organisms in a dry state when surrounded by nitrogenous or albuminous material. Chester (4) says that *Pseudomonas radicicola*, when dried in thin films on glass, perishes very rapidly, but that it may live 11 to 16 days on cotton. Harding and Prucha (23) have shown that *Bacterium campestris* may

live for as long as 13 months on cabbage seeds, but when dried on cover slips it is dead at the end of 10 days. Briscoe (1) says that this difference is no doubt largely due to the difference in the hygroscopic moisture retained by these substances. He found that tubercle bacilli lived only 8 to 12 days when dried in thin smears on glazed-paper slips. *Bacillus coli*, *B. violaceus*, and *B. prodigiosus*, according to his experiments, were even more sensitive dried under those conditions.

As to the relative merits of desiccation in room air and in a desiccator, some fairly positive statements have been obtained. Chapin (3, p. 195) says that as a rule bacteria live longer when dried in a desiccator than when dried in the open air under natural conditions. Ficker (7) showed that the rapid drying of organisms in a desiccator over calcium chlorid or sulphuric acid was preferable to drying in ordinary room air. Ficker's experiment (7), in which the organisms were placed alternately in a desiccator and a moist chamber for a couple of hours at a time, resulted in the organisms so treated dying much more rapidly than did those which were left in the desiccator continuously for the same length of time. Löhnis (15) states that frequent changes between drying and remoistening are most injurious, but that rapid drying in a space with a "rarefied atmosphere" (in a desiccator) is comparatively favorable. Unpublished experiments of J. Simon have shown that the repeated drying and moistening of the soil is much more detrimental to nodule bacteria than keeping the soil constantly dry. Chester (4), in his experiments with *P. radicicola*, found that an important condition for the successful preservation of the organism in a dry state was to keep the culture sealed from the air and in a dark, cool place.

The evidence obtainable from the literature in regard to the length of time an organism may live in air-dry soil and the factors responsible for its longevity are neither definite nor complete. Lipman (14, pp. 228 and 230) says that—

> Under air-dry conditions each soil grain is surrounded by a very thin film of moisture designated as hygroscopic water . . . According to Hall the film of hygroscopic moisture is about 0.75μ (0.00003 in.) thick . . . Nevertheless, it will be seen that the moisture, even in air-dry material, is deep enough to allow the bacteria a reasonable amount of protection. This will account for the survival of non-spore-bearing bacteria in dry soil for a long time. Indeed, instances are on record of the isolation of *Azotobacter* and *Nitrosomonas* from soils that had been kept in the laboratory for several years.

Löhnis (15, p. 67) says that—

> vegetative cells can better endure drying when they are in soil. With spores also this is true. The resistance of spores dried in earth is usually found to be higher than that of spores dried on cotton, silk, glass, etc.

Duggar and Prucha (6) found that after the rapid drying out of soil cultures there remained a large number of living organisms whose vitality

would extend over a considerable period. Nestler (16) investigated an old herbarium and found that even after 23 years 90,000 colonies could be obtained from 1 gram of soil. Azotobacter (12) remain alive in soil samples if these samples are kept for 160 days in a desiccator and then 148 days in an air-tight condition. Germano's (9) results seemed to indicate that the organisms of typhoid fever and diphtheria did not live as long in soil as on fabrics, although the diphtheria bacillus averaged 20 to 40 days' longevity in all trials in soil. Firth and Horrocks (3) found that the typhoid bacillus would live for 23 days in dry sand. Pfuhl (18) found the typhoid bacillus to live 28 days in dry sand and 88 days in moist garden earth. The bacillus of dysentery, on which he experimented at the same time, lived only 12 days in sand and 101 days in moist garden earth. Briscoe (1) found the tubercle bacillus to live 213 days in garden soil.

But little work has been done to determine the effect of different soil types on the longevity of organisms dried in them. The data offered in the literature on this point are not only scanty but far from recent. Modern texts hold that dust does not offer protection to many pathogenic organisms, the dangers due to ordinary dust being much exaggerated according to Rosenau (22, p. 72) and Chapin (3, p. 263). Dempster (5) found that the cholera vibrio lived only a short time in perfectly dry soil, but survived for a prolonged period in soil containing a small amount of moisture. The typhoid bacillus showed a greater tenacity of life in soil than did the cholera vibrio, but entire desiccation proved to be quickly fatal to it also. Comparison of the longevity of these organisms in white sand, gray sand, garden mold, and peat showed that with the exception of peat, which apparently contained substances toxic to the organisms, the nature of the soil did not have a direct influence on them. The vitality of the organisms appeared to depend rather on the moisture content of the soil than on its composition. Our experiments on the longevity of soil organisms in different types of soil have led to a modified conclusion. The longevity of vegetative cells in air-dry soil is probably, as Lipman (14, p. 228) suggests, due mainly to the presence of moisture in the hygroscopic form, although undoubtedly the presence of organic colloidal substances with a tendency to retain moisture and with other properties is of importance. Van Suchtelen, in speaking of the analysis of soil solution as quoted by Giltner (10, p. 154), makes certain statements, which, on account of their immediate bearing on this subject, deserve direct quotation. He says:

> In many cases there was found in the soil solution a slime. This must be regarded as the first experimental proof of the presence of this substance in the soil, and it is not impossible that much of the irregular behavior of the life in soil can be explained to some extent with a knowledge of this slime. If I may be permitted, I should like to call your attention to the possibility of this substance having an effect on desiccation, diffusion, and other processes.

It is the above statement which has stimulated and formed the basis of the experimental work recorded herein. No progress has been made in the direction of an explanation of the nature of this slime. Its effect on the prolongation of the life of micro-organisms subjected to desiccation has been the object in view.

EXPERIMENTAL STUDY

An experiment was conducted to determine whether an organism may receive protection from the solution in which it is suspended before being subjected to desiccation in sand. For this work were used cultures of *P. radicicola* grown for five days at room temperature on nitrogen-free ash agar. For suspension the following solutions were employed:

(1) Physiological salt solution.
(2) Physiological salt solution + 0.1 per cent of agar.
(3) Physiological salt solution + 0.1 per cent of gelatin.
(4) Physiological salt solution + 0.1 per cent of albumin.
(5) Physiological salt solution + 0.1 per cent of gum arabic.
(6) Physiological salt solution + 0.1 per cent of soluble starch.

With the exception of the albumin solution these were all prepared by dissolving 1 gm. of the dry substance in a small amount of salt solution and then making it up to a volume of 1,000 c. c. They were found to be practically neutral to phenolphthalein. On account of the difficulty of dissolving powdered egg albumin it was found necessary to use raw white of egg, a quantity being taken which by computation contained 1 gm. of albumin. As albuminous solutions may be heated to 100° without coagulation if slightly alkaline, this solution before sterilization was made $-10°$ F. S. by the addition of $N/1$ sodium hydroxid. After sterilization (which with all six was accomplished by the Tyndall method, 30 minutes heating in flowing steam on four successive days) the $N/1$ sodium hydroxid was neutralized with $N/2$ hydrochloric acid, leaving the albumin solution like the other five, practically neutral.

Suspension of the bacterial growth from four agar slopes was made in 250 c. c. of each of the above solutions. For the purpose of securing initial counts 1 c. c. of each suspension was diluted and plated on nitrogen-free ash agar. Twelve flasks of quartz sand were then inoculated from each of the six solutions, 5 c. c. to a flask. The sand had been prepared after the method described by Rahn (19). It was heated with diluted hydrochloric acid, washed several times, first with tap water and then with distilled water, heated on a water bath until almost air dry, and then heated at least 30 minutes over a free flame. Fifty gm. of the dry sand was placed in 100 c. c. Erlenmeyer flasks, which were plugged with cotton. Sterilization was accomplished by heating for 45 minutes in the autoclave under 15 pounds' pressure.

The inoculated flasks were kept in a dark, well-ventilated place at a temperature of 22° to 25° C. At intervals the number of organisms per

gram of sand was determined by the plate method, samples being taken from two flasks representing each suspension solution. Nitrogen-free ash agar was used for all plates and these were kept 10 days at a temperature of 22° to 25° C. before counting.

It is evident from Table I that the counts are irregular and not such as to form a basis for any positive conclusions. This is due in part to the fact that the fluctuations in numbers from time to time were so extreme that it was difficult to determine what dilutions should be used to obtain plates from which accurate counts might be made. One great mistake in this trial was the addition to the sand of a quantity of moisture which was sufficient to permit the multiplication of the organisms for three weeks after inoculation of the flasks. In later trials the addition of less moisture lessened the period of multiplication. The bacteria were not actually subjected to desiccation until after January 27, by which time the difference in the numbers of organisms developing on the five different substances was such that a fair comparison of their water-retaining capacity during the process of drying was not possible. Although it is true that after a desiccation period extending over almost four weeks (from the last of January to February 24) there were greater numbers of living organisms in the flasks to which the albumin solution had been added, it is possible that this would not have occurred had not the organisms in those flasks reached enormous numbers just previous to the period of drying, because of the superior nutritive qualities of this substance.

TABLE I.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gum-arabic solution.	Starch solution.	Gelatin solution.	Albumin solution.
Jan. 20[a]	60,000	60,000	60,000	60,000	60,000	60,000
27	27,400	428,700	30,000	60,500	626,400	−10,000
15	1,711,000	3,651,000	63,160	2,143,000	3,974,000	(?)
27	674,800	328,000	60,000	468,100	1,335,600	3,677,000
Feb. 13	1,000	1,000	−1,000	−1,000	10,000	30,000
24	−50	−50	50	50	−50	200

[a] Initial counts.

Another experiment of the same nature was made with the following solutions:

(1) Physiological salt solution.
(2) Physiological salt solution + 0.1 per cent of agar.
(3) Physiological salt solution + 0.1 per cent of gelatin.
(4) Physiological salt solution + 0.1 per cent of gum arabic.
(5) Nutrient broth.
(6) Milk.
(7) Soil solution (extracted from garden soil, sandy loam, by the method of Van Suchtelen).[1]

[1] All soil solutions were furnished by Mr. J. Frank Morgan, Research Assistant in Bacteriology.

The bacterial growth from one agar slope was suspended in 12 c. c. of each of the above solutions, and 1 c. c. was diluted and plated quantitatively on nitrogen-free ash agar. From each of the seven suspensions 2 c. c. was added to each of five flasks of quartz sand, which was of the same quality and prepared exactly as in the preceding trial.

These flasks were kept in a dark place at 22° to 25° C. Quantitative determinations, made at intervals, are based on plates from but a single sample of each set, consequently the opportunity for error is materially increased. It can not, therefore, be claimed that these figures (Table II) show accurate comparisons. However, it is quite evident that between March 26 and April 17, during which time the sand was so dry as to make the multiplication of organisms impossible, the rate of decrease in the numbers of organisms taken from broth, milk, and soil solution was noticeably less than that of organisms from the other solutions. This implies a certain protection gained from the presence of nitrogenous or albuminous constituents in the milk or broth. To what substance or substances in the soil solution such protection should be credited can not be stated definitely. The slime, mentioned by Van Suchtelen (10, p. 154), may be of influence in this connection.

TABLE II.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Broth.	Milk.	Soil solution.
March 18..	1, 100, 000	1, 500, 000	1, 440, 000	1, 613, 000	1, 024, 000	1, 176, 800	1, 460, 000
26..	−10, 000	−10, 000	10, 125, 000	−10, 000	19, 967, 000	185, 000	40, 000
April 6....	−25	25	50	−25	220, 000	405, 000	8, 600
17....	−25	−25	−25	−25	−25	−25	−25

An additional experiment was conducted employing the same cultures used in the previous experiments. The procedure was the same, except that as a basis for quantitative determinations two samples were taken from each set instead of one. As the plates from several of the flasks showed no colonies whatever on May 3, even in the lowest dilutions, which represented 1/25 gm., it was thought advisable in making the next determinations, on May 13, to take 1-gm. samples from these flasks and mix them directly with the melted medium in the Petri dish instead of plating 1 c. c. of a dilute suspension as previously done. It is quite evident that the direct mixture of the sand with the plating medium tends to give higher counts than those secured by plating the washings of the sand, for in the latter case a large number of organisms undoubtedly remain attached to the sand particles instead of being washed off into the suspension. This difference in technic may account for the apparent increase in numbers in certain cases, as shown by the last plating.

TABLE III.—*Longevity of Pseudomonas radicicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Albumin solution.	Broth.	Milk.	Soil solution.
April 16	1,648,000	2,144,000	1,901,000	3,234,000	360,000	1,477,000	4,026,000	1,266,000
	−25	25	−25	−25	56	428,625	515	391
May 3	.....	.....	116	.....	2	432,000	106	3,080
13	.....	.....	.....	.....	.....	.....	.....	.....

The figures in Table III offer little except a general confirmation of the results of the two other experiments. As the sand was air dry after April 26, it may be understood that the counts on May 3 and May 13 represent the numbers surviving 7 and 17 days desiccation, respectively. Attention must be called to the fact that the lack of figures to show the comparison in increase of bacteria in the different solutions between April 16 and April 26 makes it impossible to overlook entirely the function of these different solutions in their nutritive capacity. Plates were made on April 26, but the nitrogen-free agar made up with maltose instead of saccharose proved an unfortunate choice; for no colonies whatever developed, although, as seen by the two subsequent platings, living organisms were then present in abundance. However, the favorable influence of the soil solution, whether it may be as a food material for soil organisms or a protection during desiccation, can not be disputed.

An experiment was conducted to compare the longevity of *P. radicicola* dried in quartz sand and in clay-loam garden soil. As in the foregoing experiments, the organism was grown for five days at room temperature on nitrogen-free ash agar. The bacterial growth from one agar slant was transferred to 12 c. c. of physiological salt solution and the mixture shaken thoroughly, and 1 c. c. of the suspension was diluted and plated quantitatively. To the two flasks each of clay loam and quartz sand were added 2 c. c. of the bacterial suspension. The clay loam had been sifted and air dried. The quartz sand had been prepared after Rahn's method, described previously. Fifty-gm. portions of each were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and sterilized by heating in the autoclave for 45 minutes under 15 pounds' pressure.

The inoculated flasks were shaken to distribute the organisms throughout the sand or soil, and then kept in a dark, well-ventilated place at a temperature of 22° to 25° C. The number of living organisms per gram of sand and loam was determined at intervals by plating quantitatively from two samples of each.

TABLE IV.—*Difference in longevity of Pseudomonas radicicola dried in quartz sand and in clay-loam soil*

Date.	Sand.	Clay loam.
April 16	1,648,000	1,648,000
May 3	25	42,133
13	.....	33,025

It is evident from the data above tabulated that a larger number of organisms survive a limited period of desiccation in clay loam than in quartz sand. This may be partly explained by the difference in grain size and hygroscopic moisture of the two. A given weight of coarse quartz sand consisting of large particles has a surface much less than that of the same quantity of finely divided garden soil, and it therefore retains a much smaller amount of moisture in the hygroscopic form. If the grain size were the only distinction between sand and clay-loam soil, it might properly be concluded that the longevity of organisms in such materials is directly proportional to the percentage of hygroscopic water retained. Such a conclusion is not permissible, however, for the clay-loam soil differs from the sand not only in texture but in content of organic constituents. The amount of such material in any sand is small, and in this case, where the sand was treated with acid, it may be regarded as having been absent. The experiments already described indicate that the soil solution contains substances which offer to the bacteria some protection against desiccation. The soil solution used in our experiments was extracted from just such a soil as was used in the experiment now under discussion.

A further experiment was conducted to compare the changes in numbers and kinds of organisms when soil solution is dried in different types of soils. Soil solution extracted from a rich garden loam was used for this experiment. The soils, obtained from the Soil Physics Department of the Michigan Agricultural College, were of five different types: Muck, sand, sandy loam, clay, and clay loam.

Fifty-gm. portions of these soils in the air-dry condition were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and were then sterilized in the autoclave for 45 minutes under 15 pounds pressure. For greater exactness the total quantity of soil solution was agitated and then divided into five 250 c. c. portions; from each of these 1 c. c. was plated on ordinary agar in dilutions of 1 to 10,000, 1 to 100,000 and 1 to 1,000,000. Ten flasks of each type of soil were then inoculated with the soil solution, all the solution used for any one type of soil being taken from a single flask. Although it was desired to have the inoculum approximately equal in all cases, a quantity of liquid which barely moistened the muck and clay loam was found to more than saturate the coarser soils. So to make the physical conditions more nearly alike, 15 c. c. of the solution was used for each flask of clay, clay loam, and muck, but only 10 c. c. for the flasks of sand and sandy loam.

The inoculated flasks were kept on a shelf in the laboratory at a temperature of 20° to 25° C., exposed to very dim diffused light, and subject to the influence of normal variations in the humidity of the room atmosphere. At intervals of about four weeks quantitative determinations were made, samples being taken from two flasks of each soil. After the first plating, samples were taken from one flask opened at the previous

plating and from one new flask each time, the object being to secure more representative counts. Plates were made with ordinary agar and kept for one week at a temperature of 22° to 25° C. before counting.

Moisture determinations were made in duplicate at the time of each quantitative plating, the bacterial counts being then computed on the oven-dry basis.[1] Small variations in the percentage of moisture, occurring after the soils attained the air-dry condition (which with sand and sandy loam was by March 3 and with the other three soils between March 3 and March 29), are probably the result of fluctuations in the humidity of the room air. In the case of clay it was impossible to secure a thoroughly mixed sample, owing to its drying into a sort of hard, baked condition; therefore, a slight irregularity in the moisture determinations could not be avoided. The data are recorded in Table V.

TABLE V.—*Number of bacteria per gram in 50 grams of sand, sandy loam, clay, clay loam, and muck when dried after the addition of soil solution*

Date.	10 c. c. of soil solution added.				15 c. c. of soil solution added.					
	Sand.		Sandy loam.		Clay.		Clay loam.		Muck.	
	Number of bacteria per gram.	Percentage of water.	Number of bacteria per gram.	Percentage of water.	Number of bacteria per gram.	Percentage of water.	Number of bacteria per gram.	Per cent-age of water.	Number of bacteria per gram.	Percentage of water.
1914:										
Nov. 17	285,200	20.0	170,000	20.0	462,900	30.0	225,000	30.0	453,900	30.0
Dec. 29	4,318,000	14.54	26,170,000	14.38	11,500,000	28.96	60,840,000	31.81	33,089,000	26.13
1915:										
Jan. 28	1,912,000	6.25	5,806,000	2.81	1,492,000	19.17	26,006,000	16.96	16,613,000	24.85
Mar. 3	197,000	.1	1,555,000	.84	914,000	3.59	12,798,000	9.83	5,782,000	19.51
29	51,900	.36	1,967,000	.78	552,000	.93	4,659,000	2.93	4,924,000	16.33
Apr. 21	18,900	.16	1,066,000	.84	447,100	1.57	4,135,000	3.31	4,217,000	16.32
May 7	32,500	.27	983,000	1.08	278,800	1.81	3,845,000	3.65	2,220,000	16.25
14	37,000	.22	2,245,000	1.10	378,000	1.74	3,914,000	3.63	2,703,000	15.91
June 18	41,000	.20	3,218,000	1.22	494,000	1.98	5,456,000	4.26	1,836,000	16.80
Sept 6[a]	127,600	.14	6,523,000	1.23	1,241,000	2.26	11,686,000	4.30	2,781,000	16.78

[a] This count was made by Mr. O. M. Gruzit, Graduate Assistant in Bacteriology.

With a view to determining the predominant types of organisms placed in the soils, isolations were made from a few of the most common types of colonies occurring on the plates of the original soil solution. The characteristics of these organisms were studied. It must not be assumed, however, from the fact that so few organisms were isolated, that the flora of the soil solution was limited to the species observed. The high dilutions necessary for obtaining accurate quantitative plates failed, of course, to show up the organisms which were present in smaller numbers. From the quantitative plates made after the soils reached the air-dry state, between March 3 and May 7, isolations were made of the most numerous types. As the muck plates were frequently overgrown with a downy white mold, but few pure cultures could be obtained from

[1] Dried at 105° C. for 24 hours.

that source. As seen in Table V, the loam soils and muck show a higher count six months from the time of inoculation than do the clay and sand. During the first six weeks all five soils contained an amount of moisture sufficient for bacterial growth, and during the last two months only were the soils in the air-dry state. The amount of activity in the period intermediate between the optimum and minimum supply of moisture shows a gradual decrease, the rate varying in the different soils.

While there was not a great difference in the initial counts, the opportunity for bacterial growth in the five types of soil was by no means the same. This is clearly evidenced by the contrast between their counts during the first period, when the moisture content was yet sufficient to permit multiplication. Since the sand was saturated with the amount of soil solution used as an inoculum, it at first presented conditions more favorable to anaerobic than to aerobic species. As this amount of moisture diminished and the oxygen supply increased, opportunity for the growth of aerobic types was given, but the extent of this favorable period was limited not only by the small amount of organic food material but also by the extremely rapid evaporation of moisture. Conditions in the clay were at first comparable with those in the sand, it being practically waterlogged. With the gradual reduction in moisture and increase in aeration, the growth of aerobic and facultative bacteria proceeded. The smaller size of the grains produced two noticeable effects—viz, a limited oxygen supply, inhibitory to the extensive multiplication of aerobic species, and a prolonged retention of moisture, which favored the longevity, if not the activity, of non-spore-bearing bacteria. As in the sand, a low content of organic nutrients acted as a natural limit to the growth of saprophytic species. In the clay loam, sandy loam, and muck multiplication was possible from the start, for the amount of solution used for inoculation was just sufficient to moisten the soils without saturating them. Their higher content of organic substance also gave them an advantage in respect to nutrition.

However, in these soils also differences in size of grain, thickness of moisture film, and oxygen supply proved to be factors of more influence than the mere abundance of organic food substance. The muck, for instance, although containing the highest percentage of such organic materials, proved to be of a less favorable medium for bacterial growth than did the clay loam. The grain size of the clay loam appeared to be that which was most advantageous with respect to aeration, thickness of moisture film, and retention of hygroscopic water. Its content of decomposable substances, while not so great as that of the muck, was more than sufficient for microbial development. The sandy loam, with a smaller amount of organic materials, somewhat larger grain size, and consequently less hygroscopicity, did not show as large numbers of living

organisms at any time as did the clay loam, although its oxygen supply in consequence of these same conditions must have been somewhat greater.

We therefore perceive that the optimum condition for microbial activity in soil is a proper adjustment of these previously mentioned factors. With regard to longevity, fewer factors are concerned, the data so far obtained indicating that it is a function of both grain size (and therefore amount of hygroscopic moisture) and content of organic substances.

The influence of soil type was made evident not only in the numerical counts but also in the varieties of organisms persisting in the different soils throughout the two months during which they were in the air-dry state. As the condition of the sand had been such as to favor the development of organisms with high oxygen requirements, plates of high dilution always showed a predominance of those types. Such of these as were spore bearers became a larger and larger proportion of the total number, as the period of desiccation extended and the non-spore-bearing species died out. Among the spore bearers most frequently found were *Bacillus mycoides* and aerobes of similar morphological and cultural characters. Of the non-spore-formers an organism found in larger numbers than any other single species showed the greatest longevity. The characteristics of this organism are as follows:

It is a rod with rounded ends, 0.6μ by 1.3 to 1.5μ. It is actively motile, non-spore-forming and non-capsule-forming. It is frequently observed in pairs. It stains readily with aqueous alcoholic fuchsin. In nutrient broth it produces a decided turbidity, some sediment, and a soft surface scum. The growth on agar is glistening, translucent, grayish white, and very abundant. On a gelatin stab there is a white surface growth, with a filiform growth in the stab, but not liquefaction. Litmus milk becomes bluer after 48 hours; some peptonization in 30 days. No indol from Dunham's peptone solution. Ammonia produced from Dunham's solution and nitrates reduced. Facultative anaerobe. Optimum temperature, 25° C. Habitat, soil.

Physical conditions in the clay had somewhat inhibited the extensive multiplication of strongly aerobic types, but permitted the development of facultative bacteria. Since anaerobic organisms could not be secured by the method of plating used, no mention of them is possible. As the non-spore-bearing types declined, the plates showed more evidence of spore-bearing, strictly aerobic varieties similar to those met with in the sand. The fact that such colonies were not found until their diminishing numbers necessitated the use of lower dilutions suggests their development from spores which had merely remained latent in the clay without passing through a process of multiplication and subsequent destruction like the majority of the facultative non-spore-bearing species. The non-spore-forming organism showing greatest endurance of desiccation was a type identical with that persisting in the sand.

During the period of extensive multiplication, the plates from sandy loam, clay loam, and muck showed quite similar types, although the sandy loam has slightly greater numbers of the strongly aerobic spore-forming species. As the numbers diminished, spore-bearing types became more frequent on plates from both sandy loam and clay loam, but were not evident on the plates from muck. It is to be inferred that the multiplication of those in the finest soil had not progressed to such an extent as to make their colonies numerous in high dilutions, their numbers apparently being in proportion to the grain size and amount of aeration. The most persistent non-spore-bearing organism was of the type already referred to, as found in clay and sand. In addition to this, certain chromogenic cocci and one variety of slime-forming organism were frequent on plates from all three of these soils through the time of desiccation. This slime former, which was especially numerous on plates from muck, is described as follows:

The organism is a rod 0.4μ by 0.6 to 0.7μ; nonmotile. No spores observed. No capsule demonstrated. Stains readily with aqueous alcoholic fuchsin. Nutrient broth made slimy and very turbid. Growth on agar spreading, translucent, orange-yellow, slimy. Gelatin stab, surface growth and rapid liquefaction. Litmus milk discolored, alkaline, slimy; peptonization begun in 48 hours and complete in 10 days. Facultative anaerobe. No indol from Dunham's peptone solution. Ammonia produced from Dunham's solution and nitrates reduced. Habitat, soil.

Attention should be called to the rather peculiar circumstance that not one of the organisms isolated during the last two months corresponds to any one of the four organisms which predominated in the original soil solution used for the inoculation of the five soils. The extinction of these species may have been due either to the unfavorable influence of association with other organisms during the period of active multiplication or to their lack of endurance when supplied with less than the optimum amount of moisture.

CONCLUSIONS

(1) The survival of non-spore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a soil is not directly proportional to its grain size and hygroscopic moisture.

(2) Bacteria, at least those tested, resist desiccation longer in a rich clay loam than in sand, under the conditions of our experiment.

(3) If bacteria are suspended in the solution extracted from a rich clay loam before being subjected to desiccation in sand, they live longer than if subjected to desiccation after suspension in physiological salt solution.

(4) The solution extracted from a rich clay loam contains substances which have a protective influence upon bacteria subjected to desiccation.

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OBSERVATIONS ON THE LIFE HISTORY OF THE CHERRY LEAF BEETLE

By GLENN W. HERRICK, *Entomologist*, and ROBERT MATHESON, *Assistant Entomologist, Cornell University Agricultural Experiment Station*

INTRODUCTION

The cherry leaf beetle (*Galerucella cavicollis* Lec.), which attracted much attention during the season of 1915, is a native insect that has adopted several new food plants, at least in the beetle stage. Not since the first record of its work on cultivated plants, in 1894, has its injury been as great or as widespread as during the summer just past. It would seem that the early prediction of Davis (2),[1] who first recorded the beetle's work on cherry (*Prunus* spp.), was about to be fulfilled, that as it was a northern and widespread species we might expect it to become increasingly injurious from year to year.

HISTORICAL REVIEW

The cherry leaf beetle was originally described by Le Conte in 1865 (5, p. 216) from a single specimen received from North Carolina. Nothing further is recorded of this beetle till 1890, when Packard, who found this species in large numbers at Berlin Falls, N. H., eating holes in the leaves of wild cherry, probably the pin cherry (*Prunus pennsylvanica*), refers (7, p. 529) to it under the name "*Galeruca sanguinea*."

The next reference is by Davis (2), who reports it as being abundant at Bellaire, Mich., during the summer of 1894 and destroying the foliage of cultivated cherries. This is the first record of this beetle's attacking the foliage of cultivated trees, and Davis makes the suggestion that as this insect is a northern species it may yet become quite injurious. The larvæ were found in this same locality; but it is not stated on what plants they were feeding, though the writer states that wild cherries were only a short distance away.

Lintner (6) records this beetle as occurring in thousands on June 10, 1895, at Ausable Forks, N. Y., feeding on the foliage of the cherry left uninjured by late frosts. He also states that his correspondent found this same insect at work early in July on the foliage of young chestnut trees, but that he did not verify this observation.

Felt (3), in 1898, records outbreaks of this insect at Corning, N. Y., the beetles occurring in such numbers as to threaten the destruction of the trees. Smith was the first to record the occurrence of this beetle on peach, having found it in Pennsylvania during the summer of 1898.

[1] Reference is made by number to "Literature cited," p. 949.

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Johnson (4) reports an extensive outbreak on "fire cherry" (*Prunus pennsylvanica*) at Ricketts, Wyoming County, Pa., during September, 1897, the beetles and larvæ occurring in immense numbers.

Chittenden (1) reports outbreaks of this beetle in June, 1898, at St. Ignace, Mich., on cherry and at Spruce Creek, Huntington County, Mich., on young peach trees. He states that larvæ are known to feed on cherry and probably also on peach, but mentions no definite records of such occurrences on the peach.

Since the publication of Chittenden's article, nothing has been recorded of this insect, and undoubtedly during all the years since 1898 no injury of any consequence has been committed by it.

OUTBREAKS IN NEW YORK IN 1915

During the summer of 1915 several severe outbreaks occurred in New York, the beetles defoliating cherry, peach (*Amygdalus persica*), and plum (*Prunus* spp.). On June 3 Mr. E. P. Putnam, of Jamestown, N. Y., wrote the Entomological Department, inclosing specimens of beetles, saying that they were defoliating wild cherry and peach trees in the park and also reported them as seriously defoliating cherry and peach trees throughout the town and neighboring districts. On June 11, Mr. H. B. Rogers reported them as injuring cherry and peach and later wrote that this beetle had done considerable injury throughout Chautauqua County. Reports of injury have been received from the following localities: Sonyea (cherry, peach, and plum); Perry, Scio (cherry); Olean, Honeoye Falls (cherry); Bath (cherry); Holland (cherry); Collins, Gowanda, Wyoming, Batavia (cherry and peach); Perrysburg (cherry); Jamestown (cherry and peach); Chautauqua (cherry and peach); Kennedy, Fredonia, Ripley (plum and peach); Castile (cherry); Elmira (cherry and peach); Hornell (cherry); and Ithaca (cherry and peach). All these reports came during the month of June and early in July and nothing has been heard of later injury. Evidently the beetles have not bred locally in such numbers that the work of the adults would attract attention in August and September.

The causes which brought about so widespread an outbreak of this insect can not at present be determined. Practically all the injury was restricted to western and southwestern New York. It has been suggested that the beetles migrated northward from Pennsylvania, but this does not seem plausible, as the native host, *Prunus pennsylvanica*, is a northern tree, occurring southward only as far as Pennsylvania and in the mountains to North Carolina and Tennessee. Conditions must have been favorable for the increase of this beetle in 1914 and hibernation must have been attended with little loss of life, resulting in such large numbers of the overwintering beetles as to cause overcrowding of the normal food plants. Should favorable conditions prevail during any year, we may again look for a sudden and perhaps more widespread outbreak.

LIFE HISTORY AND HABITS

The cherry leaf beetle is a pretty, dull-red beetle measuring 4.5 to 5.5 mm. in length (Pl. LXIV, fig. 1). The antennæ are black, and the legs vary from almost black to nearly reddish in color. There are no strikingly distinguishing characters, but the coloring will nearly always serve to separate it from the more closely related northern species. The beetle is widely distributed, occurring from Canada through the New England States southward into Pennsylvania and west to Wisconsin. Chittenden (1) also records it from Texas and Vancouver, British Columbia. The original specimen described by Le Conte (5, p. 216) is from North Carolina.

This insect is one of our native beetles and up to 1894 had only been recorded on wild cherry. In that year it was found attacking the cultivated cherry, destroying the foliage. Later Smith (8) recorded it as injuring peach, and this year it has been reported as feeding on plum. How much more extended the feeding habits of this beetle may become can not even be guessed, though its future destructiveness will depend largely upon whether the larvæ can also adapt themselves to new and closely related food plants.

The beetles pass the winter in hibernation and, although the time of emergence has not been determined, they probably appear in May or, if the weather is favorable, during the latter part of April. They feed actively during May and June not only on the pin cherry but also on the peach, cherry, and in some instances the plum (Pl. LXV, fig. 5). In the field the beetles began to leave the cultivated food plants early in July and practically all had gone by the middle of the month.

In New York State there is only a single brood a season. The new brood of adults appears during the second week in August and becomes common during the latter part of the month and early September; they feed almost exclusively on the pin cherry and do not seem to migrate far from their host plant. In our rearing cages they began entering the soil or crawling under stones about the middle of September, but on fine days would return to feed on the pin-cherry foliage. In early October they had all entered hibernating quarters and did not leave them even on the finest or warmest days.

The work of the beetles is most noticeable during June and early July. After the middle of July the beetles had largely disappeared from the cultivated trees about Ithaca. Although many adults had been seen in copula, no eggs were observed, despite a close watch on all their new food plants. It was supposed that in accordance with the habits of closely allied species, as the elm leaf beetle (*Galer cella luteola*), the eggs would be found on the host plant.

On July 21 Mr. Cotton, a student in the Entomological Department, found adults and what he considered larvæ of this species on pin cherry.

On examination it was at once seen that there were larvæ in all stages, but the closest search did not reveal a single egg on the foliage or trunk or branches of the tree. The youngest larvæ, which seemed to us to have just hatched, were very active, running about over the trunk and branches, and a search at the base of the trees soon revealed immense numbers of eggs just below the surface of the soil, in the matted grass, under sticks, and among rubbish.

THE EGG

We did not observe the date of the first egg laying nor determine the number of eggs laid by a single female. At Ithaca egg laying occurs from June to August. If we judge from the length of the larval life and the egg stage, the deposition of eggs at Ithaca undoubtedly began the last week in June. The egg-laying period extended throughout July and the early part of August.

The egg is entirely different in shape from that of closely allied species. It is oval, of a light-straw color, and measures 0.72 to 0.84 mm. in length by 0.60 to 0.64 mm. in width. The entire surface is marked with rather regular hexagonal areas. Large numbers of these eggs were found at the base of the few pin-cherry trees located close to the Cornell University grounds. The eggs adhered rather firmly to each other and to the matted grass. Although close search was made, no eggs could be found at the base of any other species of Prunus (Pl. LXV, fig. 1, 2).

THE LARVA

During the latter part of July eggs hatched in from 14 to 18 days after they were laid. The young larva escapes from the egg by cutting a hole through one side with the mandibles. The young larvæ are very active, running about rapidly. They soon find their way to the trunk of the tree and could be seen any time during the hatching period clambering actively over the branches in search of the young and tender foliage near the tips of the twigs. They are found most commonly on the under surface of the foliage skeletonizing the leaves. They feed ravenously, grow rapidly, and reach maturity in from two to three weeks. Where the larvæ are abundant all the foliage may be so completely skeletonized as to turn brown and die, giving the trees a scorched appearance (Pl. LXV, fig. 3, 4). The length of the life cycle, with the number of molts, is given in Table I.

TABLE I.—*Length of life cycle and number of molts of the cherry leaf beetle*

Eggs.		First stage.	Second stage.	Third stage.	Entered soil to pupate.	Emergence of adult.
Laid.[1]	Hatched.					
.....	July 23	July 30	Aug. 3	Aug. 9	Aug. 10	Aug. 28
.....	...do.....	July 29	...do.....	...do.....	...do.....	Do.
.....	...do.....	July 30	Aug. 4	Aug. 8	Aug. 9	Aug. 26
.....	...do.....	July 28	Aug. 1	Aug. 4	Aug. 5	Aug. 24
.....	...do.....	July 27	...do.....	Aug. 6	Aug. 7	Aug. 25
.....	...do.....	July 29	Aug. 3	Aug. 8	Aug. 9	Aug. 27
.....	...do.....	...do.....	Aug. 1	Aug. 7	Aug. 8	Aug. 26
.....	...do.....	July 30	Aug. 2	...do.....	...do.....	Aug. 27
.....	...do.....	July 28	...do.....	...do.....	...do.....	Aug. 28

[1] From another series of experiments the length of the egg stage was determined. The eggs hatched as follows: 15, 18, 17, 18, 16, 18, and 14 days after they were laid. The average is 16 days. If this were taken as the average length of the egg stage, the total length of the life cycle from the egg to the adult would vary from 48 to 53 days.

DESCRIPTION OF LARVAL STAGE

FIRST INSTAR.—The newly-hatched larva is depressed, fuscous in color, the head, thoracic shield, legs, and anal segment, black. Scattered over the larva are a number of setæ. Length, 1.4 to 1.6 mm.; greatest width, 0.45 to 0.50 mm.

SECOND INSTAR.—Nearly cylindrical, slightly depressed, fuscous to brown in color, the head, legs, thoracic and anal shields black. The ground color is almost entirely obscured by the black areas as shown in Plate LXIV, figure 2. On each segment, except the prothoracic and anal, there are two oval, rather sharply defined, large, black areas separated from each other by a narrow line. Laterad of the black areas are angular black markings as shown in Plate LXIV, figure 2. Length, 2.5 to 3.5 mm.

MATURE LARVA, THIRD INSTAR.—Length, 6 to 8 mm., nearly cylindrical, somewhat depressed, with an average width of about 2 mm. (Pl. LXIV, fig. 3). The larva after the second molt measures 5 mm. in length and is black in color. As it feeds, the black spots and markings become separated and the brownish yellow ground color shows distinctly. Head black, narrower than thorax; mouth parts yellowish brown. Legs, prothoracic and anal shields black. Dorsally each segment, except the prothorax and anal segments, with two sharply defined oval to rectangular black areas separated by a brownish yellow line; laterad of each of these there is an angular black spot and beyond each of these a smaller rounded black mark. Along the lateral margin there is an elongate oval black spot on each segment. The venter of each abdominal segment is marked with five dark brown to black spots, the central one being largest. The prosternum is black; meso- and meta-sterna each with a narrow, elongate, black area in front and two black rounded spots just caudad of it.

FOOD HABITS OF THE LARVA

From a close examination about Ithaca we failed to find the larvæ present on any trees but the pin cherry. The few trees of this species located near the campus were swarming with the beetles and larvæ. However, on the other food plants of the adult we found, late in the season, only a few beetles and no larvæ. To determine whether the larvæ could survive and reach maturity on the other species of Prunus the following experiments were performed:

EXPERIMENT 1.—On July 23 six larvæ, some almost mature, were placed on the leaves of *Prunus avium*. Two died on July 25, two more on the 27th, and the remaining two entered the soil to pupate on July 28, the adults emerging on August 15. The immature larvæ did not feed, but the nearly mature forms fed slightly before entering the soil to pupate.

EXPERIMENT 2.—On July 23 two young larvæ were placed on leaves of *Prunus avium*. Both died on the 26th without having fed at all.

EXPERIMENT 3.—On July 27 three half-grown larvæ were placed on leaves of *Prunus virginiana*. On the 28th all had left and entered the soil in an attempt to pupate. Later all failed to pupate and died.

EXPERIMENT 4.—On July 27 five half-grown larvæ were placed on leaves of *Prunus virginiana*. On July 28 one was dead and the others entered the soil. All failed to reach maturity.

EXPERIMENT 5.—On July 28 three half-grown larvæ were placed on leaves of *Prunus serotina*. All failed to feed and died on July 31. On the same day four more half-grown larvæ were placed on leaves of *P. serotina*. All failed to feed and died on July 30.

It will be seen from the above experiments that the larvæ seem to be unable to survive on either the cultivated sweet cherry (*Prunus avium*) or the common two native varieties *P. serotina* and *P. virginiana*. It is unfortunate that through an oversight experiments were not made with the other species of Prunus. The food plants of the larvæ are undoubtedly restricted at the present time to the wild red, or pin, cherry. Whether the larva can succeed in adapting itself to other host plants seems to be a doubtful question, so that in the future the abundance of the beetles will depend not so much on the presence of its enemies as on a goodly supply of the larval food plant.

THE PUPA

Pupation takes place at or slightly below the surface of the soil. No special preparation is made by the larva, the pupa often lying openly on the surface in the grass or under rubbish. The pupa is bright yellow, strongly convex, without any distinguishing markings. Scattered over it are small, short brownish tipped setæ, which aid in preventing injury from the soil. The tip of the abdomen is furnished with two diverging strong black spines (Pl. LXIV, fig. 4).

CONTROL OF CHERRY LEAF BEETLE

On account of the comparatively small numbers of the beetles at Ithaca, we were not able to conduct control experiments. However, several of our correspondents have had good success with lead arsenate (paste) used at the rate of 4 to 5 pounds to 100 gallons of water and also with a spray containing 40 per cent nicotine. In the case of the nicotine spray our correspondent used it at the rate of 3 pints to 100 gallons of water and reported good success. He also reports failure with lead arsenate, though using treble and even quadruple the quantities generally recommended for foliage-feeding insects.

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PLATE LXIV

Galerucella cavicollis:

Fig. 1.—Adult.
Fig. 2.—Larva, second instar.
Fig. 3.—Larva, third instar.
Fig. 4.—Pupa.

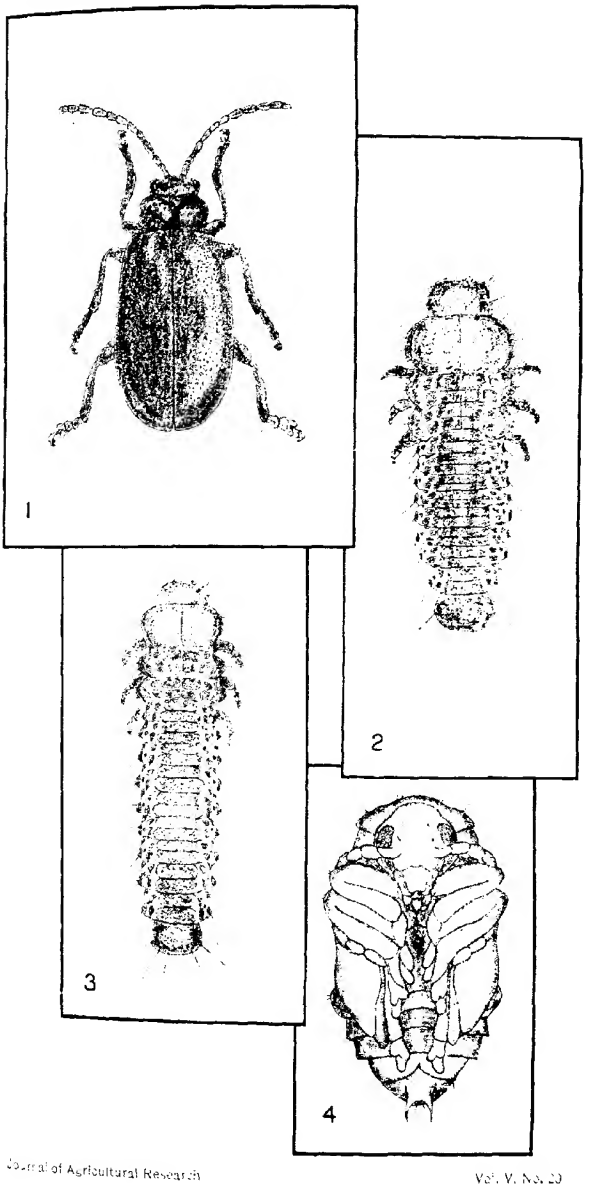
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PLATE LXV

Galerucella cavicollis:

Fig. 1.—Eggs on ground at base of tree.
Fig. 2.—Eggs, enlarged.
Fig. 3.—Larvæ feeding on leaf.
Fig. 4.—Work of larvæ on foliage.
Fig. 5.—Work of beetles on foliage.

APPARATUS FOR MEASURING THE WEAR OF CONCRETE ROADS

By A. T. GOLDBECK,

Engineer of Tests, Office of Public Roads and Rural Engineering

Many miles of concrete roads have been built during the past few years, and the methods employed in their construction are rapidly becoming standardized. The concrete mixture is now made comparatively rich, and in general the aggregates are selected with as much care as present knowledge of these materials permits. Even yet, however, it is doubtful whether the right mixture is being used for the purpose: Whether it is too rich for economy or whether it should be made still richer. It is questionable what kinds of coarse aggregates give the most economical results: Whether they should be composed of hard, tough fragments of trap rock or of softer, more friable pieces of limestone of approximately the same degree of hardness as the mortar in which they are embedded; whether angular fragments of crushed stone should be used or whether round pieces of gravel are equally satisfactory. Definite knowledge on these points based on scientific information seems to be lacking.

The ideal concrete road should wear uniformly and slowly. When due care is exercised in construction and the necessary precautions are taken in maintenance, uniformity of wear may to a large extent be controlled. But little is known about the rate of wear of concrete roads having various aggregates and carrying different kinds of traffic. General observation indicates that some roads with particular kinds of aggregates are wearing more slowly than others containing different coarse aggregates, even though the traffic conditions are nearly alike. We have, however, no definite idea of the amount of wear in these different roads. There must come a time in the life of every concrete road when, notwithstanding careful maintenance through crack protection and patching, its thickness will approach the minimum, making imperative the expenditure of a considerable amount of money for a new wearing surface to replace that gradually worn away by traffic. Every fractional part of an inch decrease in thickness therefore represents a very definite depreciation in the value of the pavement. Money can not be expended intelligently on various aggregates mixed with cement in different proportions for road construction without accurate knowledge of one of the most important factors governing the expenditure—namely, the probable rate of depreciation of the road as determined by actual wear.

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This consideration has led the Office of Public Roads and Rural Engineering to attempt to gain definite knowledge of the wear of concrete roads carrying various kinds of traffic, and a special instrument has been designed by the writer and built in this office for that purpose. Several methods of taking autographic records of the cross section of the road were considered, but were discarded in favor of the simpler and more portable form of instrument finally constructed.

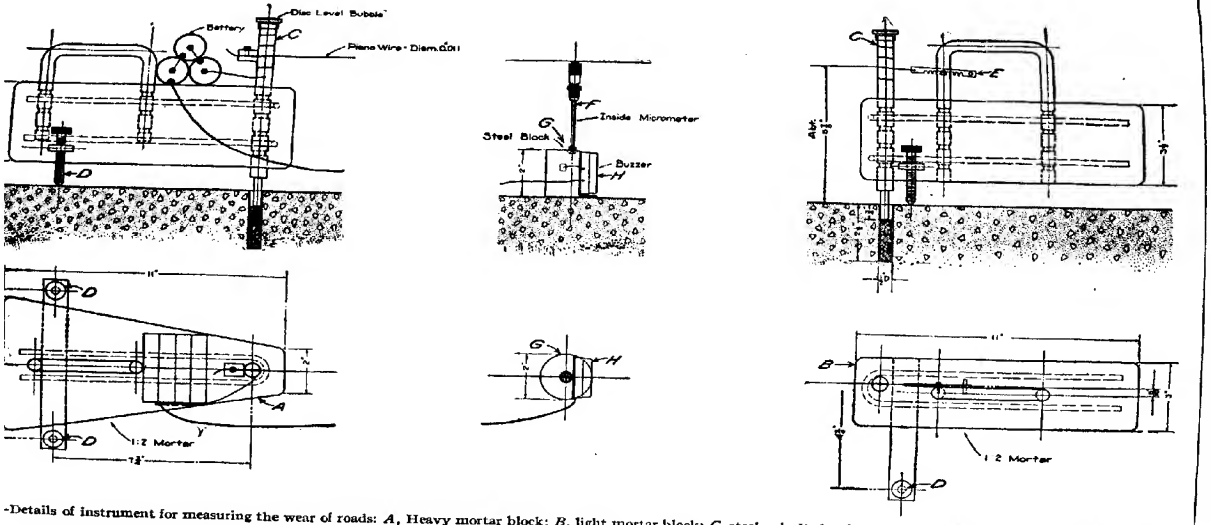
Essentially, this instrument consists of a fine wire stretched tightly across the road at a constant height, together with an inside micrometer for measuring the distance from the road surface to the wire. Measurements taken 1 foot apart across the road permit the plotting of its cross section, and if these measurements are repeated at long intervals the change of cross section or the decrease in the thickness of the road will be revealed.

The accompanying illustrations show the instrument in detail and its method of application on the road. If Plate LXVI, figure 1, and text figure 1 are first referred to, the component parts of the apparatus may be seen very plainly.

Pieces *A* and *B* are made of cement mortar and have embedded in them steel rods, *C*, drilled with holes slightly inclined with the horizontal. A fine piano wire about 0.01 of an inch in diameter is passed through these holes and is stretched across the road from block *A* to block *B*. The tops of these rods are each provided with a disk-level bubble, so that when placed in position in the road the rods may be adjusted to a vertical position. Block *A*, which is heavier than block *B*, is provided with two adjusting screws, *D*, for adjusting rod *C* to the vertical. Block *B* rests on two points only, one the lower end of rod *C* and the other the end of adjusting screw *D*. Constant tension is produced in the wire by the weight of block *B*, which is pivoted about the bottom of rod *C* and is adjusted to a horizontal position by means of rack *E*, provided at the end of the wire. As the weight of block *B* is constant, the tension in the wire, and consequently the amount of sag for like spans, must remain the same. A very definite and fixed datum is thus provided, which should remain constant from year to year and which is very easily established by merely placing the end blocks of the apparatus in their proper position on the road.

The bottoms of rods *C* are spherical in shape; and when in use on concrete roads, they rest on the flat tops of bronze plugs cemented in the road surface. These plugs are ½ inch in diameter and are 1¾ inches long. They are set ¾ inch below the surface, and their tops are protected by means of a brass pipe plugged with a bituminous-sand mixture during the long intervals between readings.

In obtaining the wear measurements a chalk line is first snapped across the road between the bronze plugs, and the points at which it is



-Details of instrument for measuring the wear of roads: *A*, Heavy mortar block; *B*, light mortar block; *C*, steel rod; *D*, level adjusting screws; *E*, adjusting rack; *F*, inside micrometer; *G*, steel bearing block; *H*, electric buzzer.

purposed to take readings are marked on this line. At these points a steel block, *G*, 2 inches in diameter, is placed, in order to avoid measuring the small local inequalities in the road surface. In the top of this block a flat-bottomed cylindrical recess is made, and an ordinary inside micrometer is held in the recess, while its upper end is adjusted to contact with the steel wire stretched across the road. An electric buzzer, *H*, is mounted on the side of this block, and when contact is made between the micrometer and the wire an electric circuit is completed through the buzzer. With this instrument readings for wear may be taken to the nearest 0.001 inch, although this degree of accuracy will not be necessary.

Holes in the road in which the bronze plugs are set are drilled by means of a special hand-operated drill press carrying a star drill.

In Plate LXVI, figure 2, the method of mounting the apparatus in the road and its manipulation are plainly shown. On the left is the heavier end block carrying the batteries, and on the right is the lighter block the weight of which supplies constant tension to the fine steel wire, part of which is seen in front of the operator. The cord extending on the road surface from the heavier block to the small steel block carrying the micrometer is one of the leads from the battery to the electric buzzer.

Placing the buzzer in this position near the operator obviously is advantageous, especially when the instrument is to be used amidst the distracting noises of traffic. The end blocks are set as near to the sides of the road as practicable, in order to permit measurements being taken across almost the entire width of the road. Should longitudinal cracks develop through the sections measured, the readings so taken will be rendered useless; and in order to eliminate this difficulty, sufficient plugs must be set to permit obtaining readings at uncracked sections.

Wear measurements of this kind taken of the actual road surface should prove of great future value if the traffic conditions and the physical characteristics of the concrete materials likewise are known, and should help to decide present moot questions regarding concrete roads and road materials. Not only may concrete surfaces be measured for wear in this manner, but the wear or vertical movement of other kinds of road surfaces may likewise be determined by the use of this instrument.

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PLATE LXVI

Fig. 1.—Photograph of details of instrument for measuring wear of roads: *A*, Heavy mortar block; *B*, light mortar block; *C*, steel rod; *D*, level adjusting screws; *E*, adjusting rack; *F*, inside micrometer; *G*, steel bearing block; *H*, electric buzzer.

Fig. 2.—Instrument in use on concrete road.

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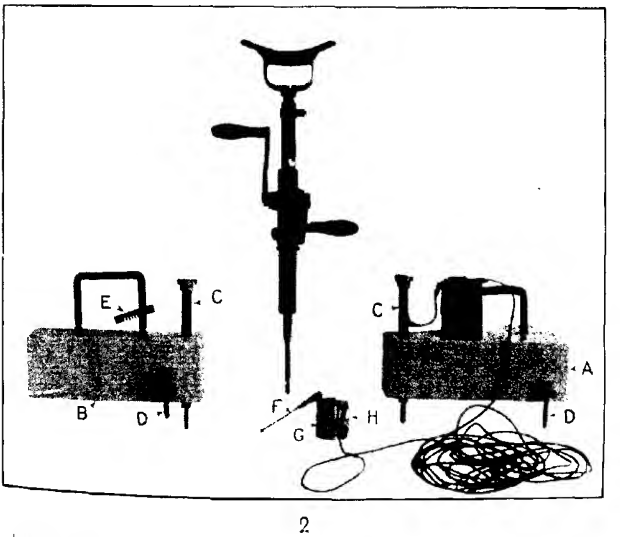

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